

In re Application of: Michal DANIELY et al  
Serial No.: 10/771,440  
Filed: February 5, 2004  
Office Action Mailing Date: July 6, 2011

Examiner: Bradley DUFFY  
Group Art Unit: 1643  
Attorney Docket: **26003**  
Confirmation No.: 3178

### **REMARKS**

Reconsideration of the above-identified Application in view of the amendments above and the remarks following is respectfully requested.

Claims 72, 73 and 82-93 are pending in this Application. Claims 72, 73, 82, 87, 89, 91 and 93 have been rejected under 35 U.S.C. §103(a). Claim 72 has been amended herewith.

#### **35 U.S.C. §103 Rejections**

##### **Bubendorf in view of Brown**

The Examiner has rejected claims 72, 73, 82, 87, 89, 91 and 93 under 35 U.S.C. §103(a) as being unpatentable over Bubendorf (Am. J. Clin. Pathol. 116: 79-86, 2001) in view of Brown (Urol. Clin. NA 27: 25-37, 2000). The Examiner states that Bubendorf teach methods of identifying transitional cell carcinoma cells or diagnosing bladder cancer from a voided urine by obtaining a voided urine sample from a subject, staining nucleated cells of the sample with Papanicolaou, imaging the nucleated cells and identifying morphological abnormality; and methods of identifying transitional cell carcinoma cells or diagnosing bladder cancer from a voided urine by obtaining a voided urine sample from a subject, staining nucleated cells of the sample with DAPI and FISH probes, imaging the nucleated cells and identifying morphological abnormalities like larger nuclear size, irregular nuclear shape and patchy, and then enumerating FISH signals on these same single cells to identify transitional cell carcinoma or diagnose bladder cancer. The Examiner states that Bubendorf does not expressly teach that transitional cell carcinoma cells can be identified by a high nucleus to cytoplasm (N/C) ratio; and that this deficiency is made up for in the teachings of Brown, who teaches other morphological abnormalities that identify transitional cells as suspicious as transitional carcinoma cells with a high nucleus to cytoplasm ratio and cells that are enlarged and identifying such abnormalities with a Papanicolaou stain. The Examiner states that it would have been a *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to predictably substitute Papanicolaou stain for the DAPI stain of

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Bubendorf to identify a high nucleus to cytoplasm ratio; that one would have appreciated that there are multiple art-recognized morphological abnormalities and multiple art-recognized stains that can identify the known morphological abnormalities of transitional cell carcinoma in voided urine samples and would have considered such art-recognized staining techniques as obvious variants which can predictably substituted one for the other; and because these stains and morphological abnormalities well all known, and because methods of imaging were known in the art, one of skill in the art would have a reasonable expectation of success in identifying transitional cell carcinoma cells from a voided urine sample. With respect to claim 93, Examiner states that Bubendorf teach that the sample should be cytospun at a density of 100-400 cells per field and optimizing to 100-200 cells per field if more than 4000 cells were in the field, thus the prior art recognized that the cell density of cells on the cytopsin was to be optimized. Examiner's rejection is respectfully traversed. Claim 72 has been amended herewith.

Neither Bubendorf nor Brown teach staining with FISH of morphologically stained cells with an intermediate step of destaining the morphologically stained cells as claimed.

For clarity, Applicants are describing the teachings of Bubendorf and Brown individually but are traversing the rejection with respect to the combination of these references, *infra*. That is, the Applicants are not attacking the references individually, rather addressing the combinations of references as set forth in the instant Office Action.

With respect to Bubendorf Applicants point that while Bubendorf et al. stain the cells with Papanicolaou they do not analyze the FISH abnormalities on the Papanicolaou-stained cells. Thus, Bubendorf specifically states:

*“One slide was fixed with SprayFix.... and stained with standard Papanicolaou. The other slides were air dried and stored at -70°C for subsequent FISH analysis”*

(Bubendorf, Page 80, left column, last paragraph; Emphasis added).

Thus, Bubendorf failed to teach the step of:

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*“staining said stained nucleated cells resultant of step (a) using fluorescent *in situ* hybridization (FISH) to thereby obtain nucleated cells stained with FISH”*

Applicants point that in order to detect the nucleus to cytoplasm ratio the nucleus and the cytoplasm should be detected and measured, however, DAPI which was taught by Bubendorf only stains the cell nucleus.

With respect to Brown, Applicants point that Brown et al. merely describe cytology analysis of voided urine using parameters such as a nucleus to cytoplasm ratio.

With respect to Examiner's assertion that in order to identify the high nucleus to cytoplasm ratio the skilled in the art would have found it obvious to substitute Papanicola stain for the DAPI stain of Bubendorf et al., Applicants point that the skilled in the art, who is well aware of the auto-fluorescence resulting from histological stains such as May-Grünwald-Giemsa, Giemsa, Papanicola or Hematoxylin-Eosin, would not have performed FISH analysis on such stained histological slides since FISH staining is viewed under a fluorescence microscope, and any auto-fluorescence of histological dyes may hamper the accuracy of the FISH analysis by obscuring, or adding noise to the FISH signals, thus risking the accuracy of identification of transitional cell carcinoma and diagnosis of bladder cancer.

In sharp contrast to the cited art, either alone or in combination, the invention as now claimed recites a de-staining step which enables the removal of the dye used in the histological staining (e.g., May-Grünwald-Giemsa, Giemsa, Papanicola and Hematoxylin-Eosin) prior to employing FISH analysis on the SAME stained cells.

Thus, the currently amended claimed invention is novel and non-obvious over the combined teachings of Budendorf and Brown since none of the cited art, nor a combination thereof teach or suggest a method of identifying transitional cell carcinoma and diagnosing bladder cancer by performing a histological staining and identifying morphological abnormalities based on the nuclear to cytoplasm ratio, and then de-staining the histological stain and performing FISH analysis and analyzing the FISH signals on the same cells that appear suspicious as being transitional carcinoma cells based on the morphological abnormality in the N/C ratio, and confirming the

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identification of transitional cell carcinoma cells based on an abnormal FISH signal on these same morphologically abnormal cells, as in the currently amended claimed invention. .

Support for claim amendment can be found in Page 26, line 2, in the instant application as filed.

***Bubendorf, Brown, US Patent 6,418,236, Kaplinsky***

The Examiner has rejected claim 84 under 35 U.S.C. 103(a) as being unpatentable over Budendorf et al., 2001, in view of Brown (2000) as applied to claims 72, 73, 82, 87, 89, 91 and 93 above, and further in view of U.S. Patent 6,418,236 (Ellis, 2002) or Kaplinsky (2001). The Examiner states that Budendorf in view of Brown teach and render obvious the methods as set forth in the above rejection; that these references do not expressly teach imaging cells with an automated imaging device capable of dual imaging; and that this deficiency is made up for in the teachings of U.S. Patent 6,418,234 or Kaplinsky. The Examiner states that U.S. Patent 6,418,234 teaches automated image analysis using a microscope capable of dual imaging to image cells stained with two stains such as morphological stains and *in situ* hybridization stains which can be fluorescent stains; that Kaplinsky teaches automated image analysis using a microscope capable of dual imaging to image the same cells stained with a morphological stain and fluorescent *in situ* hybridization stains; and that it would have been *prima facie* obvious to one of ordinary skill in the art to identify transitional cell carcinomas cells from a voided urine sample using the processes rendered obvious by Bubendorf in view of Brown and additionally imaging the stained cells with an automated microscope capable of dual imaging to identify the same single cells as transitional cell carcinoma cells; that one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success because the stains and the processes of Bubendorf and Brown were known in the art to identify transitional cell carcinoma cells while the automated imaging microscope of U.S. 6,418,236 would allow for faster processing of samples. Examiner's rejection is respectfully traversed. Claim 72 has been amended herewith.

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Applicants point that the currently amended claims, which recite the “*de-staining step*” between the histological staining by May-Grünwald-Giemsa, Giemsa, Papanicolaou or Hematoxylin-Eosin and the subsequent FISH staining on the SAME cells, are novel and inventive over the combined art of Bubendorf and Brown, even when using a microscope capable of dual imaging as described in U.S. Patent No. 6,418,236, or the microscope capable of dual imaging of Kaplinsky, since none of the cited art, nor a combination thereof teach or suggest a method of identifying transitional cell carcinoma and diagnosing bladder cancer by performing a histological staining and identifying morphological abnormalities based on the nuclear to cytoplasm ratio, and then de-staining the histological stain and performing FISH analysis and analyzing the FISH signals on the same cells that appear suspicious as being transitional carcinoma cells based on the morphological abnormality in the N/C ratio, and confirming the identification of transitional cell carcinoma cells based on an abnormal FISH signal on these same morphologically abnormal cells as in the currently amended claimed invention.

In view of the above arguments and remarks Applicants believe to have overcome the 35 U.S.C. §103(a) rejections.

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In view of the above amendments and remarks it is respectfully submitted that 72, 73, 82, 84, 87, 89, 91 and 93 are now in condition for allowance. A prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

**/Jason H. Rosenblum/**

Jason H. Rosenblum  
Registration No. 56,437  
Telephone: 718.246.8482

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**Enclosure:**

- Petition for Extension of Time (One Month)